Asian Journal of Chemistry Vol. 15, No. 1 (2003), 155–159

Chromatographic Studies on Triphala

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Chromatographic studies on Triphala, a well known ayurvedic formulation, prepared from three constituents, *viz.*, Haritaki, Beheda and Amalaki, have been carried out with a view to standardize the formulation. Thin layer chromatographic studies were carried out using precoated silica gel fluorescent plates on the aqueous extracts and ethyl acetate extracts of the three constituents and also Triphala. The choice of the solvent system toluene-ethyl acetate-formic acid is discussed and its utility is demonstrated for complete resolution of gallic acid from other components. Gallic acid therefore can be used as a marker compound for standardizing Triphala.

Key words: TLC, Triphala, Gallic acid, Standardization.

INTRODUCTION

Triphala, a well-known ayurvedic formulation, is being used for centuries and its uses are well documented in the ayurvedic literature. The chemistry of the three constituents or Triphala, viz., Haritaki (*Terminalia chebula*), Beheda (*Terminalia bellerica*) and Amalaki (*Emblica officinalis*) has been extensively studied. The present work on Triphala is undertaken from the point of view of standardization of the formulation. In recent years the use of herbal medicines is increasing rapidly both nationally and internationally and it can be assumed that this trend will continue as the concept of standardization takes firm roots for ayurvedic formulations. The literature survey[1–4] shows that a good deal of work is being done to develop the standardization methods for herbal medicines.

Thin layer chromatography (TLC) is very useful in the studies of herbal medicines for the obvious reasons of simplicity, cost effectiveness and speed. On most of the occasions, a good qualitative fingerprint profile of a herbal preparation serves the purpose of identification and forms an important step in quality control process. The new Ayurvedic Pharmacopoeia[5] gives methods for characterization of the crude drugs Haritaki, Beheda and Amalaki based on macroscopic and microscopic characteristics, water soluble extractives, alcohol soluble extractives, ash value, acid insoluble ash, etc. Thin layer chromatographic analysis of these extracts will be an additional and important parameter for chararacterization based on chemical constituents.

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EXPERIMENTAL

Thin layer chromatography analysis was performed on precoated aluminum plates from Merck India [20 × 20 cm silica gel 60 F_{254}, layer thickness 0.2 mm], gallic acid, tannic acid, methanol, toluene, ethyl acetate, formic acid, ferric chloride and hydrochloric acid of AnalaR grade were used. Ferric chloride solution prepared (3%) in methanol. Haritaki, Beheda, Amalaki powders were procured from local ayurvedic agencies. Triphala, a mixture of these powders in 1 : 1 : 1 proportion, was prepared as reference standard and market samples of Triphala were analyzed for comparative studies with reference standard.

Procedure: 2 g of each powder was soaked overnight in 30 mL distilled water. The water extracts were filtered and the filtrate was made up to 100 mL with distilled water. It was divided into two parts. One part was extracted with ethyl acetate and then dried, while the other part of the aqueous extract was evaporated as such. The residues from the ethyl acetate extracts and aqueous extracts were reconstituted in methanol for spotting on precoated TLC plates with microcapillaries. Gallic acid and tannic acid solutions in methanol were spotted on the same plates as controls. The R_f values of various spots were noted after observing the plates under UV-254 nm, as also after spraying with ferric chloride solution.

RESULTS AND DISCUSSION

The constituents of Haritaki, Beheda and Amalaki as mentioned in Ayurvedic Pharmacopoeia[5] are tannins and polyphenolic compounds; gallic acid, tannic acid and glycosides and ascorbic acid and gallotannins respectively. However, recent work of Ghosal *et al.*[6] shows complete absence of L-ascorbic acid in Amalaki. In view of this, a number of solvent systems were formulated in the present work for achieving good separation of phenolic acids, polyphenolic and gallotannins. Thin layer chromatography on silca gel is an adsorption process and the migration of a substance is dependent on the strength of the interactions between the adsorbent and the polar groups present. Primarily the nature of the polar groups determines the strength of these interactions; the stronger the interaction, the slower the migration. The analytes of interest in the present work being natural products which are acidic in nature, the solvent systems to be formulated should have reasonably higher solvent strength and selectivity to maximize separation. These solvent systems are further modified by addition of formic acid or acetic acid. The addition of acids to mobile phases partly deactivates the adsorbent surface and decreases tailing of polar sample components[7]. A number of such solvent systems are reported in the literature[8-11] for gallic acid, tannic acid, etc. In TLC optimizing a separation depends on the selection of the best developing solvent by adjusting solvent strength and selectivity. However, the change in solvent strength as a function of the volume per cent of more polar component is not a linear function[7], hence any such change introduced in the mobile phase needs to be tested to see its performance and this results in generating many solvent systems from the same mobile phase constituents, having different volume per cent of more polar component. In the present work different combinations of toluene and ethyl acetate with formic acid were tried and ultimately two systems—toluene-ethyl acetate-formic acid, 3 : 5 : 1 and 6 : 3 : 1 were found to give excellent resolution. The TLC data (R_f values) are

given in Tables 1 and 2 and the corresponding TLC plates of ethyl acetate extracts are shown in Figs. 1 and 2. The data show that in both the systems gallic acid is completely resolved from all other tannins and also shows its distinct presence in tannic acid. The TLC profiles of all the extracts show practically similar patterns. Since gallic acid is completely resolved from all the other spots it may be used as a marker compound for standardizing Triphala. It may also be used for the purpose of quantitative estimation if HPTLC scanner/densitometer is available for scanning at 254 nm. However, quantitative estimation is generally done using high performance liquid chromatography technique[1]. Our work on HPLC in this direction is in progress and will be reported in due course.

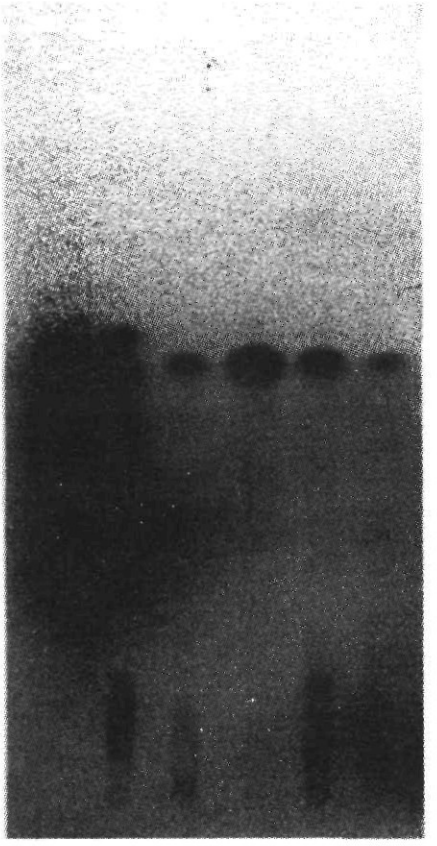
TABLE-1
R_f VALUES IN SOLVENT SYSTEM: TOLUENE-ETHYL ACETATE-FORMIC ACID (3 : 5 : 1)

Gallic acid	Tannic acid	AAE	BAE	HAE	TAE std	TAE market	AEE	BEE	HEE	TEE std	TEE market
	0.83										
0.78	0.77	0.77	0.80	0.77	0.78	0.78	0.78	0.79	0.79	0.80	0.78
	0.73										
	0.66						0.66				
	0.56	0.58	0.57	0.57	0.60	0.57	0.57	0.56	0.57	0.59	0.58
		0.50						0.47			0.48
		0.30	0.34	0.34		0.31	0.31	0.33	0.33		
				0.22	0.27	0.22	0.21	0.28			0.25
	0.18	0.17	0.16		0.18				0.19	0.17	
				0.11		0.10	0.11	0.11	0.11		0.11

TABLE-2
R_f VALUES IN SOLVENT SYSTEM: TOLUENE-ETHYL ACETATE-FORMIC ACID (6 : 3 : 1)

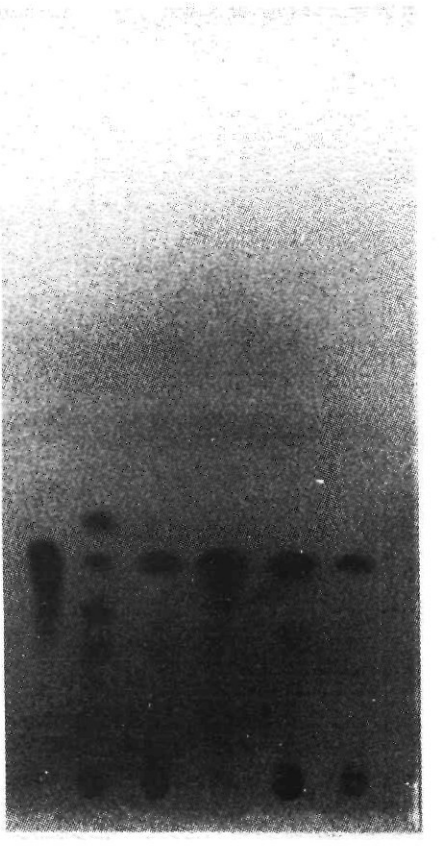
Gallic acid	Tannic acid	AAE	BAE	HAE	TAE std	TAE market	AEE	BEE	HEE	TEE std	TEE market
								0.60			
								0.53			
	0.45	0.40					0.40	0.45			
0.33	0.34	0.35	0.36	0.36	0.38	0.34	0.36	0.35	0.34	0.36	0.35
	0.27	0.26	0.25	0.25	0.26	0.26	0.27		0.25	0.27	0.25
	0.13	0.11	0.11			0.16	0.16				0.11

AAE: Amala—aqueous extract, BAE: Beheda—aqueous extract, HAE: Hirada—aqueous extract, TAE std: Triphala (std)—aqueous extract, TAE market: Triphala (market sample)—aqueous extract, AEE: Amala—ethyl acetate extract, BEE: Beheda—ethyl acetate extract, HEE: Hirada—ethyl acetate extract, TEE std: Triphala (std)—ethyl acetate extract, TEE market: Triphala (market sample)—ethyl acetate extract.



1 2 3 4 5 6

Fig. 1. Plate Developed in solvent System: Toluene-Ethyl Acetate-Formic Acid (3 : 5 : 1).



1 2 3 4 5 6

Fig. 2. Plate Developed in solvent System: Toluene-Ethyl Acetate-Formic Acid (6 : 3 : 1).

1. Gallic Acid 2. Tannic Acid 3. AEE-Amala Ethyl Acetate Extract 4. BEE-Beheda Ethyl Acetate Extract 5. HEE-Hirada Ethyl Acetate Extract 6. TEE std-Triphala (std) Ethyl Acetate Extract.

ACKNOWLEDGEMENTS

Authors' thanks are due to Prof. A.S. Bhave, Head, Dept. of Chemistry, S.P. College, Pune for providing necessary facilities. One of us (ASB) is grateful for the award of late Dr. T.R. Ingle Fellowship.

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(*Received*: 23 May 2002; *Accepted:* 5 August 2002) AJC-2811